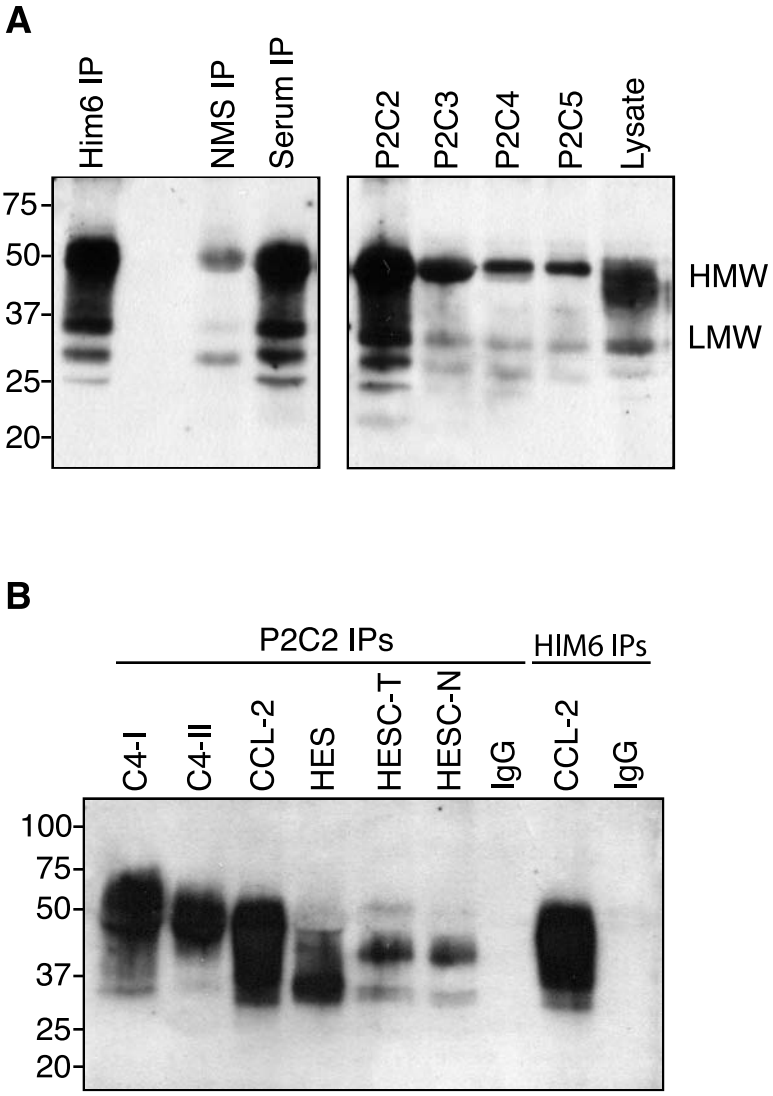
Figure Legends (Supplemental)

Supplemental Fig. 1. Development and characterization of a monoclonal antibody (P2C2) that specifically recognizes the native form of human basigin. *A*: (left panel) Serum from rBSG immunized mice was compared to the commercial antibody HIM6 mAb for their ability to IP native, glycosylated basigin proteins from CCL-2 cell lysates. NMS = normal mouse serum. Immunoprecipitated proteins were detected by immunoblot using the R&D Systems anti-hEMMPRIN polyclonal antibody and the Santa Cruz anti-Goat Hrp-conjugated secondary antibody (anti-human and anti-mouse depleted). *A*: (right panel) Identification of the basigin antibodies that recognize native glycosylated basigin. Basigin IPs from CCL-2 cell lysates were performed using supernatants from rBSG hybridoma cultures (P2C2-P2C5). Results from 4 of the 69 immunoreactive hybridoma cultures are shown. The 'Lysate' lane contains 20 μg of soluble CCL-2 lysate. The clone P2C2 was chosen for expansion and production of antibodies using a bioreactor. *B*: The basigin monoclonal antibody P2C2 immunoprecipitates glycosylated basigin proteins from a variety of human cell lines. C4-I and C4-II are human cervical carcinoma cell lines, and HES is a human uterine epithelial cell lysate. HESC-T and HESC-N refer to HESC uterine fibroblast cell lysates prepared using either the detergent Triton X-100 (T) or NP-40 (N) respectively. Control P2C2 and HIM6 IgG samples are indicated as 'IgG' and contain 5μg of each purified IgG as a control for secondary antibody cross-reactivity. Immunoblotting was performed using the anti-hEMMPRIN pAb, and the variation in the relative sizes of the proteins reflects differences in the degree of N-glycosylation of basigin in these cell lines. HMW = high molecular weight glycosylated basigin-2. LMW = low molecular weight glycosylated basigin-2.

Supplemental Fig. 2. Reciprocal basigin-neutravidin precipitations from rBSG-labeled CCL-2 cells confirms that rBSG interacts with basigin-2 and basigin-3. Biotin label transfer from rBSG to CCL-2 cells (HeLa) was performed by treating cells with increasing concentrations of SBED-labeled rBSG (0, 0.05, 0.5, 5, and 50 μg/ml) for 10 minutes at 37°C. Following 5 minutes of UV light treatment, cells were washed twice with PBS and total cell lysates collected using IP buffer. Proteins from labeled cells were precipitated with either neutravidin beads (Panels A,B) or immunoprecipitated with the P2C2 mAb (Panels C,D). Precipitated proteins were resolved by 15% SDS-PAGE in duplicate, and immunoblotted with either Streptavidin-HRP (Panels A,C) or with the R&D Systems anti-hEMMPRIN pAb (Panels B,D). The Santa Cruz goat anti-rabbit (anti-mouse, anti-human depleted)-HRP secondary antibody used for this experiment was previously shown to not cross-react with the P2C2 mAb (see supplemental figure 1B). The basigin-2 band is indicated with an arrowhead and the basigin-3/rBSG band is indicated with an arrow. This experiment was performed twice with similar results.

Supplemental Figure 1



Supplemental Figure 2